

U.S. DEPARTMENT OF COMMERCE
National Technical Information Service

PB-296 801

Nitrobenzene Ambient Water Quality Criteria

(U.S.) Environmental Protection Agency, Washington, DC

1978

BIBLIOGRAPHIC INFORMATION

PB-296 801

Nitrobenzene: Ambient Water Quality Criteria.

1978

PERFORMER: Environmental Protection Agency, Washington, DC.
Criteria and Standards Div.

Section 304(a) of the Clean Water Act (33 U.S.C. 1314(a)), requires EPA to publish and periodically update water quality criteria. These criteria are to reflect the latest scientific knowledge on the identifiable effects of pollutants on public health and welfare, aquatic life, and recreation. This report presents water quality criteria for nitrobenzene. It presents concentration criteria for the protection of fresh water and saltwater aquatic life. It presents 'safe' concentrations for humans, and in the case of suspect or proven carcinogens, gives various levels of incremental cancer risk. A section 304(a) water quality criterion is a qualitative or quantitative estimate of the concentration of a water constituent or pollutant in ambient waters which, when not exceeded, will ensure a water quality sufficient to protect a specified water use. Under the Act a criterion is a scientific entity, based solely on data and scientific judgement. It does not reflect consi, ...

KEYWORDS: *Water quality, *Toxicology, *Nitrobenzenes,
*Toxic substances, *Water pollution
effects(Humans), *Benzene/nitro.

Available from the National Technical Information Service,
Springfield, Va. 22161

PRICE CODE: PC A05/MF A01

101
PB 296801

NITROBENZENE

Ambient Water Quality Criteria

Criteria and Standards Division
Office of Water Planning and Standards
U.S. Environmental Protection Agency
Washington, D. C. 20460

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U. S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

CRITERION DOCUMENT

NITROBENZENE

CRITERIA

Aquatic Life

For nitrobenzene the criterion to protect freshwater aquatic life as derived using the Guidelines is 480 $\mu\text{g/l}$ as a 24-hour average and the concentration should not exceed 1,100 $\mu\text{g/l}$ at any time.

The data base for saltwater aquatic life is insufficient to allow use of the Guidelines. The following recommendation is inferred from toxicity data for freshwater organisms.

For nitrobenzene the criterion to protect saltwater aquatic life as derived using procedures other than the Guidelines is 53 $\mu\text{g/l}$ as a 24-hour average and the concentration should not exceed 120 $\mu\text{g/l}$ at any time.

Human Health

For the prevention of adverse effects due to the organoleptic properties of nitrobenzene in water, the criterion is 30 $\mu\text{g/l}$.

Introduction

Nitrobenzene is produced for industrial use by the nitration of benzene with nitric and sulfuric acids. Estimates of annual nitrobenzene production range from 200 to over 700 million pounds (Dorigan and Hushon, 1976; Lu and Metcalf, 1975). The principal use of nitrobenzene is for reduction to aniline, which is widely used as an ingredient for dyes, rubber, and medicinals (McGraw-Hill, 1971; Kirk and Othmer, 1967). The commercial applications of nitrobenzene are: reduction to aniline (97 percent), solvent for Friedel-Crafts reaction, metal polishes, shoe black, perfume, dye intermediates, crystallizing solvent for some substances, and as a combustible propellant (Dorigan and Hushon, 1976).

Nitrobenzene is stored in closed containers and is not usually released to the open air. Atmospheric contamination is usually prevented in plants manufacturing or using nitrobenzene by the use of activated charcoal absorbers or a carbon dioxide blanket. There is no industrial monitoring of nitrobenzene in the atmosphere. The greatest loss of nitrobenzene during production (estimated as eight million pounds annually) occurs at the acid extraction step in the purification of the crude reaction mixture, when nitrobenzene is lost to the effluent wash (Dorigan and Hushon, 1976). Thus, the greatest exposure to nitrobenzene occurs inside plants and most cases of chronic nitrobenzene exposure in man are nitrobenzene workers. Today plant levels of nitrobenzene are usually kept below the threshold limit value (TLV) of 5 mg/m^3 (Goldstein, 1975; Am. Conf. Gov. Ind. Hyg., 1977) but much

higher levels have been reported in the past (Pacseri and Magos, 1958). Nitrobenzene may also form spontaneously in the atmosphere from the photochemical reaction of benzene with oxides of nitrogen.

Nitrobenzene, also known as nitrobenzol, essence of mirbane, and oil of mirbane, is a pale yellow oily liquid with an almond-like odor (Kirk and Othmer, 1967). The color of the liquid varies from pale yellow to yellowish brown depending on the purity of the compound (Kirk and Othmer, 1967). In the solid state it forms bright yellow crystals. Nitrobenzene, $C_6H_5NO_2$, has a molecular weight of 123.11 g.

The physical properties of nitrobenzene are as follows: a boiling point of 210° to $211^\circ C$ at 760 mm Hg, a melting point of $6^\circ C$, a density of 1.205 at $15^\circ C$, a refractive index of 1.5529, and a flash point of $89^\circ C$ (Stecher, 1968). It is steam volatile (Stecher, 1968) and at $25^\circ C$ nitrobenzene has a vapor pressure of 0.340 mm Hg (Jordan, 1954).

Nitrobenzene is miscible with most organic solvents, such as ethanol, diethyl ether, acetone, and benzene (Kirk and Othmer, 1967). It is slightly soluble in water, 0.1 per 100 parts of water (1,000 mg/l) at $20^\circ C$ (Kirk and Othmer, 1967). In aqueous solutions, nitrobenzene has a sweet taste (Kirk and Othmer, 1967).

Nitrobenzene undergoes substitution reactions but requires more vigorous conditions than does benzene. Substitution takes place at either the meta (3) position or the ortho-(2) para-(4) positions depending on the physical conditions

(Kirk and Othmer, 1967). Nitrobenzene undergoes photoreduction when irradiated with ultraviolet light in organic solvents that contain abstractable hydrogen atoms (Barltrop and Bunce, 1968).

Nitrobenzene is a fairly strong oxidizing agent (Kirk and Othmer, 1967; Millar and Springfield, 1966). Since the compound can act as an oxidizing agent in the presence of aqueous solutions of alkali hydroxides, it has the capability of oxidizing compounds containing free phenolic hydroxyl groups without effectively changing these groups (Millar and Springfield, 1966). Nitrobenzene is reactive and will undergo nitration, halogenation, and sulfonation by the same methods used for benzene. However, these reactions are unlikely to occur in environmental conditions.

The reduction of nitrobenzene to aniline probably outranks all other uses of nitrobenzene as an industrial chemical (Kirk and Othmer, 1967). The di- and the trinitrobenzenes are used in military and industrial explosives. The great toxicity of nitrobenzene impairs its usefulness as an organic solvent. It is readily absorbed by contact with the skin, inhalation of the vapor, or by ingestion. The absorption of nitrobenzene into the body produces cyanosis (Kirk and Othmer, 1967).

Nitrobenzene has been found to have a metabolic turnover slow enough to result in distinct accumulation under conditions of daily exposure (Piotrowski, 1967). Complications could arise from the possible accumulation of either nitro-

benzene or p-nitrophenol. The half-life of the excretion of the p-nitrophenol is approximately 60 hours (Salmowa, et al. 1963).

The toxicological data on the effects of nitrobenzene are limited primarily to mammalian, especially human, studies and case histories. There are few data on the toxic effects of nitrobenzene to aquatic organisms. A freshwater fish acute value for nitrobenzene was found to be 42,600 $\mu\text{g/l}$ with a chronic value of more than 16,000 $\mu\text{g/l}$. A saltwater acute value was 58,539 $\mu\text{g/l}$. In the case of mammals, nitrobenzene is highly toxic when ingested, inhaled, or absorbed through the skin. Exposure by any of these routes can result in headaches, drowsiness, nausea, vomiting, and methemoglobinemia with cyanosis.

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AQUATIC LIFE TOXICOLOGY*

FRESHWATER ORGANISMS

Introduction

Static tests with the bluegill, Daphnia magna, and the alga, Selenastrum capricornutum, indicate little difference in sensitivity with no 50 percent effect concentrations lower than 27,000 µg/l. An embryo-larval test with the fathead minnow demonstrated no adverse effects at the highest test concentration.

Acute Toxicity

The 96-hour LC50 for the bluegill is 42,600 µg/l (Table 1) and, after adjustment for test methods and species sensitivity, this result provides a Final Fish Acute Value of 6,000 µg/l. The Final Invertebrate Acute Value (1,100 µg/l), based on a 48-hour EC50 of 27,000 µg/l for Daphnia magna, is lower and, therefore, it becomes the Final Acute Value.

*The reader is referred to the Guidelines for Deriving Water Quality Criteria for the Protection of Aquatic Life [43 FR 21506 (May 18, 1978) and 43 FR 29028 (July 5, 1978)] in order to better understand the following discussion and recommendation. The following tables contain the appropriate data that were found in the literature, and at the bottom of each table are the calculations for deriving various measures of toxicity as described in the Guidelines.

Chronic Toxicity

No adverse effects were observed during an embryo-larval test with the fathead minnow at test concentrations of nitrobenzene as high as 32,000 $\mu\text{g/l}$ (Table 3). After division by the sensitivity factor (6.7) a Final Fish Chronic Value of greater than 2,400 $\mu\text{g/l}$ is obtained. This also becomes the Final Chronic Value since there are no reported results with invertebrate species and EC50 results with an alga are higher. A criterion may be derived in the absence of a precise chronic value by using 0.44 times the Final Acute Value (1,100 $\mu\text{g/l}$) at which concentration no adverse effects were observed with the fathead minnow embryo-larval test.

Plant Effects

The 96-hour EC50 values for reduction of cell numbers and inhibition of chlorophyll a in the alga, Selenastrum capricornutum are 42,800 and 44,100 $\mu\text{g/l}$, respectively (Table 4).

Residues

No measured steady-state bioconcentration factor (BCF) is available for nitrobenzene. A BCF can be estimated using the octanol-water partition coefficient of 71. This coefficient is used to derive an estimated BCF of 15 for aquatic organisms that contain about 8 percent lipids. If it is known that the diet of the wildlife of concern contains a significantly different lipid content, an appropriate adjustment in the estimated BCF should be made.

CRITERION FORMULATION

Freshwater-Aquatic Life

Summary of Available Data

The concentrations below have been rounded to two significant figures.

Final Fish Acute Value = 6,000 $\mu\text{g/l}$

Final Invertebrate Acute Value = 1,100 $\mu\text{g/l}$

Final Acute Value = 1,100 $\mu\text{g/l}$

Final Fish Chronic Value = greater than 2,400 $\mu\text{g/l}$

Final Invertebrate Chronic Value = not available

Final Plant Value = 43,000 $\mu\text{g/l}$

Residue Limited Toxicant Concentration = not available

Final Chronic Value = greater than 2,400 $\mu\text{g/l}$

$0.44 \times \text{Final Acute Value} = 480 \mu\text{g/l}$

The maximum concentration of nitrobenzene is the Final Acute Value of 1,100 $\mu\text{g/l}$ and the 24-hour average concentration is 0.44 times the Final Acute Value. No important adverse effects on freshwater aquatic organisms have been reported to be caused by concentrations lower than the 24-hour average concentration.

CRITERION: For nitrobenzene the criterion to protect freshwater aquatic life as derived using the Guidelines is 480 $\mu\text{g/l}$ as a 24-hour average and the concentration should not exceed 1,100 $\mu\text{g/l}$ at any time.

Table 1. Freshwater fish acute values for nitrobenzene (U.S. EPA, 1978)

Organism	Bioassay Method*	Test Conc. **	Time (hrs)	LC50 (µg/l)	Adjusted LC50 (µg/l)
Bluegill, <u>Lepomis macrochirus</u>	S	U	96	42,600	23,300

* S = static

** U = unmeasured

Geometric mean of adjusted values = $23,300 \mu\text{g/l}$ $\frac{23,300}{3.9} = 6,000 \mu\text{g/l}$

Table 2. Freshwater invertebrate acute values for nitrobenzene (U.S. EPA, 1978)

Organism	Bioassay Method	Test Conc. **	Time (hrs)	LC50 (ug/l)	Adjusted LC50 (ug/l)
Cladoceran, <u>Daphnia magna</u>	S	U	48	27,000	22,900

* S = static

** U = unmeasured

Geometric mean of adjusted values = $22,900 \text{ ug/l}$ $\frac{22,900}{21} = 1,100 \text{ ug/l}$

Table 3. Freshwater fish chronic values for nitrobenzene (U.S. EPA, 1978)

Organism	Test*	Limits (ug/l)	Chronic Value (ug/l)
Fathead minnow, <u>Pimephales promelas</u>	E-L	>32,000	>16,000

* E-L = embryo-larval

Geometric mean of chronic values = $\frac{16,000}{6.7} = >2,400 \text{ } \mu\text{g/l}$

Lowest chronic value = $>16,000 \text{ } \mu\text{g/l}$

Table 4. Freshwater plant effects for nitrobenzene (U.S. EPA, 1978)

<u>Organism</u>	<u>Effect</u>	<u>Concentration</u> <u>(ug/l)</u>
Alga, <u>Selenastrum</u> <u>capricornutum</u>	EC50 96-hr chlorophyll <u>a</u>	44,100
Alga, <u>Selenastrum</u> <u>capricornutum</u>	EC50 96-hr cell numbers	42,800

Lowest plant value = 42,800 ug/l

SALTWATER ORGANISMS

Introduction

Static acute tests with the sheepshead minnow and Mysidopsis bahia indicate the latter is much more sensitive to nitrobenzene. Adverse effects were observed on the saltwater alga at concentrations slightly higher than the LC50 for the mysid shrimp.

Acute Toxicity

The 96-hour LC50 value for the sheepshead minnow is 58,539 $\mu\text{g/l}$ and, after adjustment for test methods and species sensitivity, this results in the Final Fish Acute Value of 8,700 $\mu\text{g/l}$ (Table 5).

As stated earlier, the mysid shrimp is more sensitive with a 96-hour LC50 of 6,676 $\mu\text{g/l}$ (Table 6). The Final Invertebrate Acute Value derived from this test is 120 $\mu\text{g/l}$; this concentration also becomes the Final Acute Value.

Chronic Toxicity

No chronic tests have been reported on the adverse effects of nitrobenzene on saltwater organisms.

Plant Effects

The cell numbers of Skeletonema costatum were reduced by 50 percent at a concentration of 9,650 $\mu\text{g/l}$ (Table 7). Chlorophyll a was equally inhibited at a concentration of 10,300 $\mu\text{g/l}$. The lower of these two results is the Final Plant Value.

Residues

No measured steady-state bioconcentration factor (BCF) is available for nitrobenzene. A BCF can be estimated using the octanol-water partition coefficient of 71. This coefficient is

used to derive an estimated BCF of 15 for aquatic organisms that contain about eight percent lipids. If it is known that the diet of the wildlife of concern contains a significantly different lipid content, an appropriate adjustment in the estimated BCF should be made.

CRITERION FORMULATION

Saltwater-Aquatic Life

Summary of Available Data

The concentrations below have been rounded to two significant figures.

Final Fish Acute Value = 8,700 $\mu\text{g/l}$

Final Invertebrate Acute Value = 120 $\mu\text{g/l}$

Final Acute Value = 120 $\mu\text{g/l}$

Final Fish Chronic Value = not available

Final Invertebrate Chronic Value = not available

Final Plant Value = 9,700 $\mu\text{g/l}$

Residue Limited Toxicant Concentration = not available

Final Chronic Value = 9,700 $\mu\text{g/l}$

$0.44 \times \text{Final Acute Value} = 53 \mu\text{g/l}$

No saltwater criterion can be derived for nitrobenzene using the Guidelines because no Final Chronic Value for either fish or invertebrate species or a good substitute for either value is available.

Results obtained with nitrobenzene and freshwater organisms indicate how a criterion may be estimated.

For nitrobenzene and freshwater organisms 0.44 times the Final Acute Value is less than the Final Chronic Value which is derived from an embryo-larval test with the fathead minnow. Therefore, it seems reasonable to estimate a criterion for nitrobenzene and saltwater organisms using 0.44 times the Final Acute Value.

The maximum concentration of nitrobenzene is the Final Acute Value of 120 $\mu\text{g/l}$ and the estimated 24-hour average concentration

is 0.44 times the Final Acute Value. No important adverse effects on saltwater aquatic organisms have been reported to be caused by concentrations lower than the 24-hour average concentration.

CRITERION: For nitrobenzene the criterion to protect saltwater aquatic life as derived using procedures other than the Guidelines is 53 $\mu\text{g}/\text{l}$ as a 24-hour average and the concentration should not exceed 120 $\mu\text{g}/\text{l}$ at any time.

Table 5. Marine fish acute values for nitrobenzene (U.S. EPA, 1978)

Organism	Bioassay Method*	Test Conc.**	Time (hrs)	LC50 (ug/l)	Adjusted LC50 (ug/l)
Sheepshead minnow, <i>Cyprinodon variegatus</i>	S	U	96	58,539	32,004

* S = static

** U = unmeasured

Geometric mean of adjusted values = $32,004 \times \frac{32,004}{3.7} = 8,700 \text{ ug/l}$

Table 6. Marine invertebrate acute values for nitrobenzene (U.S. EPA, 1978)

Organism	Bioassay Medium	Test Conc. **	Time (hrs)	LC50 (ug/l)	Adjusted LC50 (ug/l)
Mysid shrimp, <u>Mysidopsis bahia</u>	S	U	96	6,676	5,654

* S = static

** U = unmeasured

Geometric mean of adjusted values = $5,654 \text{ ug/l}$ $\frac{5,654}{49} = 120 \text{ ug/l}$

Table 7. Marine plant effects for nitrobenzene (U.S. EPA, 1978)

<u>Organism</u>	<u>Effect</u>	<u>Concentration</u> <u>($\mu\text{g/l}$)</u>
Alga, <u>Skeletonema costatum</u>	EC50 96-hr cell numbers	9,650
Alga, <u>Skeletonema costatum</u>	EC50 96-hr chlorophyll <u>a</u>	10,300

Lowest plant value = 9,650 $\mu\text{g/l}$

NITROBENZENE

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NITROBENZENE

Mammalian Toxicology and Human Health Effects

EXPOSURE

Introduction

Nitrobenzene, a pale yellow liquid at room temperature with a characteristic bitter almond aroma, is also known as oil of mirbane, nitrobenzol, and artificial bitter almond oil. It is produced for industrial use by the nitration of benzene with nitric and sulfuric acids. Estimates of annual nitrobenzene production range from 200 to over 700 million pounds (Dorigan and Hushon, 1976; Lu and Metcalf, 1975). The principal use of nitrobenzene is for reduction to aniline, which is widely used as an ingredient for dyes, rubber, and medicinals. The commercial applications of nitrobenzene are: reduction to aniline (97 percent), solvent for Friedel-Crafts reaction, metal polishes, shoe black, perfumes, dye intermediates, crystallizing solvent for some substances, and as a combustible propellant (Dorigan and Hushon, 1976).

Nitrobenzene is stored in closed containers and not usually released to the open air. Atmospheric contamination is usually prevented in plants manufacturing or using nitrobenzene by the use of activated charcoal absorbers or a carbon dioxide blanket. There is no industrial monitoring of nitrobenzene in the atmosphere. The greatest loss of nitrobenzene during production (estimated as eight million pounds

annually) occurs at the acid extraction step in the purification of the crude reaction mixture, when nitrobenzene is lost to the effluent wash (Dorigan and Hushon, 1976). Thus the greatest exposure to nitrobenzene occurs inside plants and most cases of chronic nitrobenzene exposure in man are nitrobenzene workers. Today plant levels of nitrobenzene are usually kept below the threshold limit value (TLV) of 5 mg/m^3 (Goldstein, 1975; TLV, 1978) but much higher levels have been reported in the past (Pacseri and Magos, 1958). Nitrobenzene may also form spontaneously in the atmosphere from the photochemical reaction of benzene with oxides of nitrogen; the symptoms of nitrobenzene poisoning are similar to the symptoms experienced by victims of Japanese photochemical smog (Dorigan and Hushon, 1976).

Nitrobenzene can be detected for monitoring purposes by colorimetric reaction, or by collection on a charcoal filter, extraction, reduction to aniline, and production of a colored product by diazotization of the aniline. These methods can detect 1.0 to 500 mg/m^3 (0.2 to 100 ppm) of nitrobenzene (Dorigan and Hushon, 1976). Nitrobenzene in waste water can be measured by gas chromatography (Austern, et al.) 1975). Exposure of workers to nitrobenzene is monitored by urinary levels of p-nitrophenol (Piotrowski, 1967) and p-aminophenol (Pacseri and Magos, 1958).

The liquid nitrobenzene has a very low solubility in water, although it is a good organic solvent. It also has a low volatility, does not readily react with light, and is non-corrosive. Nitrobenzene has a low volatility but a high

specific gravity, so that the fumes can accumulate at floor level in production plants. Some of the common derivatives of nitrobenzene (besides aniline) are dinitrobenzene, nitrobenzene-sulfonic acid, and nitrochlorobenzene. There are many other derivatives of nitrobenzene, and many of them are very hazardous to man as toxic agents, mutagens, and carcinogens.

Some of the physical and chemical properties of nitrobenzene are summarized in Table 1.

TABLE 1

Properties of Nitrobenzene (Dorigan and Hushon, 1976)

Formula:	$C_6H_5NO_2$ or  -NO ₂
Molecular weight:	123.11
Freezing point:	5.6 - 5.7°C
Boiling point:	210.9°C at 760 torr
Water solubility:	0.1 - 0.2 gm/100 ml at 20°C 1.0 gm/100 ml at 100°C
Soluble in:	ethanol, diethyl ether, acetone, benzene, lipids
Vapor pressure:	0.284 mmHg at 25°C 600 mmHg at 200°C
Vapor density:	4.24 (air = 1.0)
Log partition co-efficient:	hexane/water - 3.18 at 24.4°C
Density:	1.199 gm/ml at 25°C
Flash point:	87.8°C
Autoignition temp:	482.2°C
Fire hazard (N.F.P.A.):	medium; fire can be extinguished by water, foam, CO ₂ , or dry chemicals
Viscosity:	1.682 cp at 30°C
Detection level of characteristic bitter almond odor:	10 ⁻⁴ mmoles/l

Ingestion from Water

Nitrobenzene can be released into waste water from production plants as the result of losses during the production of nitrobenzene, aniline, and dyestuffs. The solubility of nitrobenzene is low, and it produces a detectable odor in water at a concentration as low as 0.03 mg/l (Austern, et al. 1975), so that large amounts can not readily accumulate unnoticed. Levels of nitrobenzene in waste water are monitored by plants producing and using the chemical but nitrobenzene levels in city water systems are usually too low to measure (Pierce, 1979). Nitrobenzene in water from an industrial spill is removed by treatment with activated charcoal.

There are no data available on mammalian toxicity of nitrobenzene ingested in drinking water.

Ingestion from Foods

There are reports of nitrobenzene poisoning resulting from its uses as false almond oil in baking, rubbing on the gums to ease toothache, contamination of alcoholic drinks, and contamination of food (Nabarro, 1948). Leader (1932) reported a case of nitrobenzene poisoning in a child who was given "oil of almonds" for relief of a cold. Acute nitrobenzene poisoning has occurred from ingestion of denatured alcohol (Donovan, 1920; Wirtschafter and Wolpaw, 1944). These cases are typical of accidental nitrobenzene ingestion. Nitrobenzene is not an approved food additive (Dorigan and Hushon, 1976).

A bioconcentration factor (BCF) relates the concentration of a chemical in water to the concentration in aquatic

organisms, but BCF's are not available for the edible portions of all four major groups of aquatic organisms consumed in the United States. Since data indicate that the BCF for lipid-soluble compounds is proportional to percent lipids, BCF's can be adjusted to edible portions using data on percent lipids and the amounts of various species consumed by Americans. A recent survey on fish and shellfish consumption in the United States (Cordle, et al. 1978) found that the per capita consumption is 18.7 g/day. From the data on the 19 major species identified in the survey and data on the fat content of the edible portion of these species (Sidwell, et al. 1974), the relative consumption of the four major groups and the weighted average percent lipids for each group can be calculated:

<u>Group</u>	<u>Consumption (Percent)</u>	<u>Weighted Average Percent Lipids</u>
Freshwater fishes	12	4.8
Saltwater fishes	61	2.3
Saltwater molluscs	9	1.2
Saltwater decapods	18	1.2

Using the percentages for consumption and lipids for each of these groups, the weighted average percent lipids is 2.3 for consumed fish and shellfish.

No measured steady-state bioconcentration factor (BCF) is available for nitrobenzene, but the equation " $\text{Log BCF} = 0.76 \text{ Log } P - 0.23$ " can be used (Veith, et al. Manuscript) to estimate the BCF for aquatic organisms that contain about eight percent lipids from the octanol-water partition coefficient (P). Based on an octanol-water partition coefficient

of 71, the steady-state bioconcentration factor for nitrobenzene is estimated to be 15. An adjustment factor of $2.3/8.0 = 0.2875$ can be used to adjust the estimated BCF from the 8.0 percent lipids on which the equation is based to the 2.3 percent lipids that is the weighted average for consumed fish and shellfish. Thus, the weighted average bioconcentration factor for nitrobenzene and the edible portion of all aquatic organisms consumed by Americans is calculated to be $15 \times 0.2875 = 4.3$.

Inhalation

Nitrobenzene is readily absorbed through the lungs with retention of up to 80 percent (Piotrowski, 1967). There are reports of nitrobenzene poisoning from inhalation of an exterminator spray for bedbugs which was sprayed on a child's mattress (Stevenson and Forbes, 1942; Nabarro, 1948). Poisonings have also resulted from inhaled nitrobenzene used as a scent in perfume and soap (Dorigan and Hushon, 1976). Chronic and acute poisoning from exposure to nitrobenzene fumes in production plants are well documented (Dorigan and Hushon, 1976; Browning, 1950; Zeligs, 1929; Hamilton, 1919), but since nitrobenzene is also absorbed through the skin, industrial poisoning cannot be attributed to inhalation alone. A worker exposed to the TLV for nitrobenzene of 5 mg/m^3 would absorb 18 mg/day through the lungs (Piotrowski, 1967).

Dermal

Nitrobenzene is highly fat-soluble and can be absorbed through the skin at rates as high as $2 \text{ mg/cm}^2/\text{hr}$ (Dorigan and Hushon, 1976). Medical literature contains many reports

of poisonings from absorption of nitrobenzene in shoe dyes and laundry marking ink. These reports were common during the 19th century and the first half of this century.

Poisoning following the wearing of newly-dyed wet shoes was reported in 1900 (Levin, 1927). The poisoning can result from nitrobenzene or aniline, both of which were used in shoe dyes and which cause the same toxic symptoms. There have been reports of cases of shoe dye poisoning in an army camp (Levin, 1927), in children who were given freshly dyed shoes (Zeitoun, 1959; Graves, 1928; Levin, 1927), and in adults. Generally the affected people are brought to the physician's attention with symptoms of dizziness, bluish color of lips and nails (cyanosis), headache, and sometimes coma. All these symptoms are due to methemoglobin formation from the absorbed nitrobenzene or aniline.

Cyanosis and poisoning of newborns who came in contact with diapers or pads containing marking ink were very common. Generally this occurred when the diapers or pads were freshly stamped by the hospital laundry (Etteldorf, 1951; Ramsay and Harvey, 1959; MacMath and Apley, 1954; Zeligs, 1929; Rayner, 1886). Often the imprint of the ink could be seen on the infant's skin. Removal of the diaper or pad and thorough washing of the skin usually reduced toxic symptoms, although methylene blue and ascorbic acid have also been used to relieve cyanosis. The toxicity is often more severe in premature infants who are in an incubator and surrounded by fumes as well as the dye on the cloth (Etteldorf, 1951). Washing

of the marked diapers or pads before their use removes the hazard of absorption of nitrobenzene or aniline from the ink.

In Egypt, "pure bitter almond oil" (a mixture of two to ten percent nitrobenzene and 90 to 98 percent cottonseed oil) has been rubbed on babies to remove crusts from the skin and to protect the children from other diseases. Zeitoun (1959) reports cases of nitrobenzene poisoning seen in Alexandria hospitals as a result of this practice.

Hamilton (1919) reported a case of chronic nitrobenzene poisoning in a woman who used it as a cleaning fluid for many years. The continuous dermal absorption caused her to experience symptoms of multiple neuritis, extreme indigestion and hemorrhages of the larynx and pharynx.

Dermal absorption of nitrobenzene is the cause of many of the chronic and acute toxic effects seen in nitrobenzene workers (inhalation also accounts for industrial toxicity although the routes of exposure often cannot be distinguished). The amount of cutaneous absorption is a function of the ambient concentration, the amount of clothing worn, and the relative humidity (high humidity increases absorption) (Dorigan and Hushon, 1976). A worker exposed to the TLV of 5 mg/m³ could absorb up to 25 mg per day; one-third of that amount would pass through the skin of a clothed man (Piotrowski, 1967). Pacseri and Magos (1958) measured ambient nitrobenzene in industrial plants and found levels of up to eight times the current TLV.

Hamilton (1919) reported a case of acute, fatal, nitrobenzene poisoning that resulted from a soap factory worker spilling "oil of mirbane" on his clothes. Immediate removal of the contaminated clothing would probably have prevented his death.

There are reports of acute and chronic poisoning due to skin absorption of dinitrobenzene by workers in munitions and nitrobenzene plants. Dinitrobenzene is believed to be much more toxic than nitrobenzene (Malden, 1907). Ishihara, et al. (1976) reported a case of poisoning where a worker handled a cleaning mixture containing 0.5 percent dinitrobenzene. The worker wore gloves, but the dinitrobenzene penetrated through the gloves to cause acute symptoms of methemoglobinemia and hemolytic jaundice. Rejsek (1947) described dinitrobenzene diffusion through the skin of munitions workers. Some of these workers who had chronic dinitrobenzene poisoning experienced an acute crisis after exposure to sun or drinking alcohol (beer). Alcohol ingestion or chronic alcoholism can also lower the lethal or toxic dose of nitrobenzene (Dorigan and Hushon, 1976). This acute reaction could occur as late as six weeks after toxic symptoms disappeared.

Although there are many literature references dealing with occupational exposure to nitrobenzene, there are few, if any, of nitrobenzene exposure resulting from water intake. Therefore, data derived from occupational exposure were employed to develop information for establishing the water quality criterion in this document.

PHARMACOKINETICS

Absorption

Nitrobenzene absorption can occur by all possible routes, but it takes place mainly through the respiratory tract and skin. At the TLV of 5 mg/m^3 , a nitrobenzene worker can absorb 18 mg/day through the lungs and 7 mg/day through the skin (Piotrowski, 1967). On the average, 80 percent of the nitrobenzene vapors are retained in the human respiratory tract (Piotrowski, 1977).

Nitrobenzene, as liquid and vapor, will pass directly through the skin. The rate of vapor absorption depends on the air concentration, ranging from 1 mg/hr at 5 mg/m^3 concentration to 9 mg/hr at 20 mg/m^3 . Air temperature does not affect the absorption rate, but an increase of relative humidity from 33 to 67 percent will increase the absorption rate by 40 percent. Work clothes reduce cutaneous absorption of nitrobenzene vapors by 20 percent (Piotrowski, 1977).

Maximal cutaneous absorption of liquid nitrobenzene is 0.2 to $3\text{ mg/cm}^2\text{/hr}$ depending on skin temperature. Elevated skin temperature will increase absorption. Absorption will decrease with duration of contact. Cutaneous absorption can be significant in industry, since contamination of skin and clothes of dye manufacture workers may reach levels of 2 and 25 mg/cm^2 , respectively (Piotrowski, 1977).

In view of this high level of absorption from lungs and skin, individual protection of exposed workers is justified.

Distribution

Upon entry into the body, nitrobenzene enters the blood

stream. Here it reacts with the hemoglobin to form its oxidation product, methemoglobin. Methemoglobin has a reduced affinity for oxygen, and the reduced oxygen carrying capacity of the blood is the cause of most of the toxic effects of nitrobenzene, including its lethality. Methemoglobin levels from nitrobenzene have ranged from 0.6 gm/100 ml in industrial chronic exposure to 10 gm/100 ml in acute poisoning (Pacseri and Magos, 1958; Myslak, et al. 1971). The normal methemoglobin level is 0.5 gm/100 ml. Under normal conditions methemoglobin will slowly be reduced to oxyhemoglobin, the normal form of blood hemoglobin.

Pacseri and Magos (1958) have demonstrated that sulfhemoglobin is also formed in the blood after chronic exposure to nitrobenzene. They found average sulfhemoglobin levels in nitrobenzene workers of 0.27 gm/100 ml (compared to the upper limit of normal of 0.18 gm/100 ml). Pacseri postulated that since blood sulfhemoglobin disappears more slowly than methemoglobin, it is a more sensitive indicator of nitrobenzene exposure. Sulfhemoglobin may be more specific than sensitive because methemoglobin is normally found in the blood but sulfhemoglobin is not.

Uehleke (1964) measured the velocity of methemoglobin formation from nitrobenzene in cats. He found the rate to be variable and not related to the blood concentration of nitrobenzene, although the methemoglobin formation velocity was maximal in each animal at the time of highest blood concentration of nitrobenzene. He also found that metabolites of nitrobenzene are able to oxidize hemoglobin. Methemoglobin formation from nitrobenzene has also been demonstrated

in vitro (Dorigan and Hushon, 1976, cited from von Oettingen, 1941; Kusumoto and Nakajima, 1970).

Further indications of the presence of nitrobenzene in the blood are the production of hemolytic anemia after acute exposure (Harrison, 1977) and the alteration of the sodium and potassium permeability of erythrocytes by derivatives of nitrobenzene (Cooke, et al. 1968).

Nitrobenzene is very lipid soluble, with an oil to water partition coefficient of 800. In a rat study the ratio of concentration of nitrobenzene in adipose tissue versus blood in internal organs and muscle was approximately 10:1 one hour after an intravenous dose (Piotrowski, 1977). Rabbits intubated with 0.25 ml of nitrobenzene had 50 percent of the compound accumulated unchanged in tissues within two days after the intubation (Dorigan and Hushon, 1976).

Dresbach and Chandler (1918) have shown cerebellar disturbance in dogs and birds from nitrobenzene vapors, although they found blood changes as described above to be the predominant effects in other mammals they tested. A histologic study attributed these effects to changes in the Purkinje cells of the cerebellum. Reports of the effect of nitrobenzene on the liver vary from description of liver damage from accumulated nitrobenzene (Dorigan and Hushon, 1976) to the statement that nitrobenzene does not cause severe renal nor liver damage (Goldstein, 1975). Goldwater (1947) has described hyperplasia of the erythropoietic centers of the bone marrow in workers chronically exposed to nitrobenzene, but he

concluded that the hyperplasia is a secondary result of the hemolytic effect of the compound. Makotchenko and Akhmetov (1972) observed secretory changes of the adrenal cortex of guinea pigs given nitrobenzene every other day at a dose of 0.2 gm/kg for six months.

Metabolism

Available information on nitrobenzene metabolism is based on animal experiments and fragmentary human data. There are two main metabolic pathways: 1) reduction to aniline followed by hydroxylation to aminophenols, and 2) direct hydroxylation of nitrobenzene to form nitrophenols. Further reduction of nitrophenols to aminophenols may also occur (Piotrowski, 1977). The rate of nitrobenzene metabolism is independent of the dose in later stages of acute or chronic intoxication. This can cause its accumulation in highly lipid tissues (Dorigan and Hushon, 1976).

The reduction of nitrobenzene to aniline occurs via the unstable intermediates, nitrosobenzene and phenyl hydroxylamine, both of which are toxic and have pronounced methemoglobinemic capacity. The reactions occur in the cytoplasmic and microsomal fractions of liver cells by the nitroreductase enzyme system (Fouts and Brodie, 1957). This enzyme system is active in mice, guinea pigs, and rabbits, and is less active in rats and dogs. The aniline is then excreted as an acetyl derivative or hydroxylated and excreted as an aminophenol. Reddy, et al. (1976) showed that the gut flora

of rats was needed for the reduction of nitrobenzene and subsequent methemoglobin formation.

The hydroxylation of nitrobenzene to nitrophenols does not occur in the microsomal fraction. The reaction proceeds via peroxidase in the presence of oxygen (Piotrowski, 1977).

Robinson, et al. (1951) studied nitrobenzene metabolism in the rabbit using ^{14}C labeled material. The main metabolic product found was p-aminophenol (35 percent) which was formed via phenylhydroxylamine. Seven phenols and aniline were detected as metabolites within 48 hours of a dose of 150 to 200 mg/kg body weight of nitrobenzene. Nitrobenzene was retained somewhat in the rabbits; its metabolites were detected in urine one week after dosing. Little unchanged nitrobenzene was excreted in the urine. The major urinary metabolites were p-aminophenol, nitrophenols, and nitro-catechol. These constituted 55 percent of the urinary metabolites and were excreted conjugated with sulfuric and glucuronic acids. About one percent of the dose was expired as radiolabeled carbon dioxide.

Yamada (1958) studied nitrobenzene metabolism in rabbits in a three-month subcutaneous exposure study. He found that urinary excretion of detoxification products varied in the early stage of exposure, but did not in the later stages. The reduction and hydroxylation pathways all became depressed during the later stages of this chronic poisoning study.

Parke (1956) reports metabolites of nitrobenzene isolated four to five days after administering 0.25 mg/kg orally as a single dose in the rabbit (see Table 2).

TABLE 2

Metabolic Fate of a Single Oral Dose (0.25 g/kg.) of [^{14}C] Nitrobenzene in the Rabbit During 4-5 Days After Dosing (Parke, 1956)

Metabolite	Percentage of Dose (average)	
Respiratory CO_2	1	} 2 in expired air
Nitrobenzene	0.6*	
Aniline	0.4+	
o-Nitrophenol	0.1	} 58 in urine
m-Nitrophenol	9	
p-Nitrophenol	9	
o-Aminophenol	3	
m-Aminophenol	4	
p-Aminophenol	31	
4-Nitrocatechol	0.7	
Nitroquinol	0.1	
p-Nitrophenyl Mercapturic acid	0.3	
(Total urinary radioactivity)	(58)	
Metabolized nitrobenzene in feces	9§	} 60 total
Metabolized nitrobenzene in tissues	15-20	
Total accounted for	85-90%	

* 0.5% in the expired air and <0.1% in the urine.

+ 0.3% in the urine and <0.1% in the expired air.

§ 6% of the dose was present in the feces as p-aminophenol.

An investigation of the metabolism of ^{14}C nitrobenzene in the cattle tick, Boophilus microplus, and spider, Nephia plumipes, was done by Holder and Wilcox (1973). They found that the tick metabolized nitrobenzene to nitrophenol and aniline whereas no free phenols were found as metabolites in the spider. Aniline was the major metabolic product in both species.

Nitrobenzene in water can be degraded by some bacteria, such as Azobacter agilis, if present in sufficiently small

amounts. Nitrobenzene tends to inhibit its own degradation at concentrations above 0.02 to 0.03 mg/l (Dorigan and Hushon, 1976; Lu and Metcalf, 1975).

Lu and Metcalf (1975) studied nitrobenzene in a model aquatic ecosystem to assess biodegradation and biomagnification. The ecosystem consisted of green filamentous algae, Oedogonium cardiacum, snails, Physa, water fleas, Daphnia magna, mosquito larvae, Culex quinquifasciatus, and mosquito fish, Gambusia affinis, under controlled atmospheric conditions. 0.005 to 0.5 mg/m³ (0.01 to 0.1 ppm) of ¹⁴C-labeled nitrobenzene was added to the water and animals were removed for analysis after 24 to 48 hours. The radiolabeled metabolites were extracted and separated by thin layer chromatography. The distribution of nitrobenzene and its degradation products is listed in Table 3.

TABLE 3

Distribution of Nitrobenzene and Degradation Products in Model Aquatic Ecosystem (Lu and Metcalf, 1975)

	R _f ^a	H ₂ O	Nitrobenzene equivalents, ppm				
			Oedogonium (alga)	Daphnia (daphnia)	Culex (mosquito)	Physa (snail)	Gambusia (fish)
Total ¹⁴ C		0.53755	0.0690	0.1812	0.5860	0.6807	4.9541
Nitrobenzene	0.72	0.50681	0.0162	0.0709	0.3952	0.3886	4.0088
Aniline	0.60	0.01262	0.0032	0.0079	0.0272	0.0169	0.2963
Acetanilide	0.35	0.00180	0.0160	-	0.0272	0.0169	0.3527
Aminophenols ^b	0.20	0.00106	0.0080	0.0315	-	-	0.0986
Nitrophenols ^b	0.10	0.00466	0.0016	0.0394	0.1226	0.2190	0.0847
Polar	0.0	0.00896	0.0240	0.0315	0.0138	0.0393	0.1130
Unextractable		0.00164	-	-	-	-	-

^a TLC with benzene:acetone:Skellysolve B (bp 60-68°C):diethylamine=65:25:25:5 (v/v).^b The isomers could not be separated reliably because of small amounts and similar R_f values.

Nitrobenzene was neither stored nor ecologically magnified. It was reduced to aniline in all organisms, acetylated in fish and water extracts only, and hydroxylated to nitrophenols by mosquito larvae and snails. The metabolites of nitrobenzene formed by the different organisms are illustrated in Figure 1.

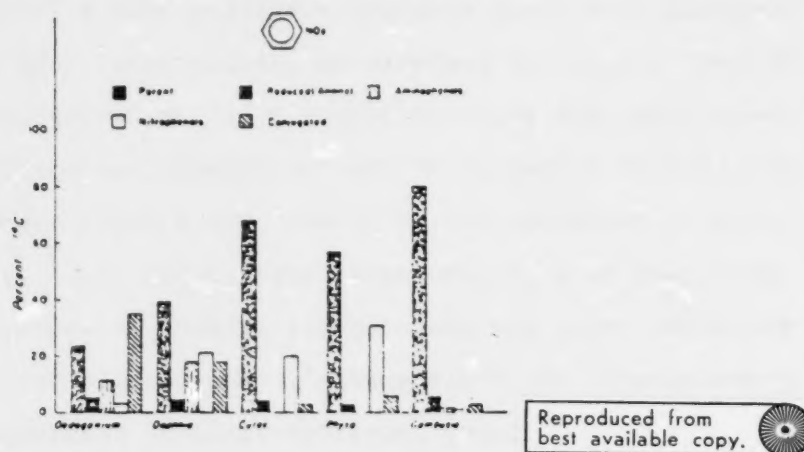


Figure 1: Relative detoxication capacities of key organisms of a model aquatic ecosystem following treatment with radioactive nitrobenzene (Lu and Metcalf, 1975).

Excretion

In man the primary known excretion products of nitrobenzene are p-aminophenol and p-nitrophenol which appear in the urine after chronic or acute exposure. In experimental inhalation exposure to nitrobenzene, p-nitrophenol was formed with the efficiency of 6 to 21 percent. The efficiency of

p-aminophenol formation is estimated from observation of acute poisoning cases where the molar ratio of excreted p-nitrophenol to p-aminophenol is two to one, since p-aminophenol is not formed at a detectable level in short subacute exposure (Piotrowski, 1977).

Ikeda and Kita (1964) measured the urinary excretion of p-nitrophenol and p-aminophenol in a patient admitted to a hospital with toxic symptoms resulting from a 17-month chronic industrial exposure to nitrobenzene. The results of their study are shown in Figure 2, which demonstrates that the rate of excretion of the two metabolites parallels the level of methemoglobin in blood. The authors exposed five adult rats to a nitrobenzene vapor of 125 mg/m^3 (25 ppm) for eight hours and measured the subsequent excretion of p-aminophenol and p-nitrophenol. The results are shown in Figure 3. The urinary excretion ratio of p-aminophenol and p-nitrophenol corresponded to their findings in the human case.

Studies of nitrobenzene concentrations in the blood of an acutely exposed person indicate that the compound remains in the human body for a prolonged period of time. Similar observations have been made from excretion of the two urinary metabolites in patients treated for acute or subacute poisoning. The excretion coefficient of urinary p-nitrophenol, followed for three weeks, is about 0.008 per hour. Metabolic transformation and excretion of nitrobenzene in man is slower by an order of magnitude than in rats or rabbits (Piotrowski, 1977).

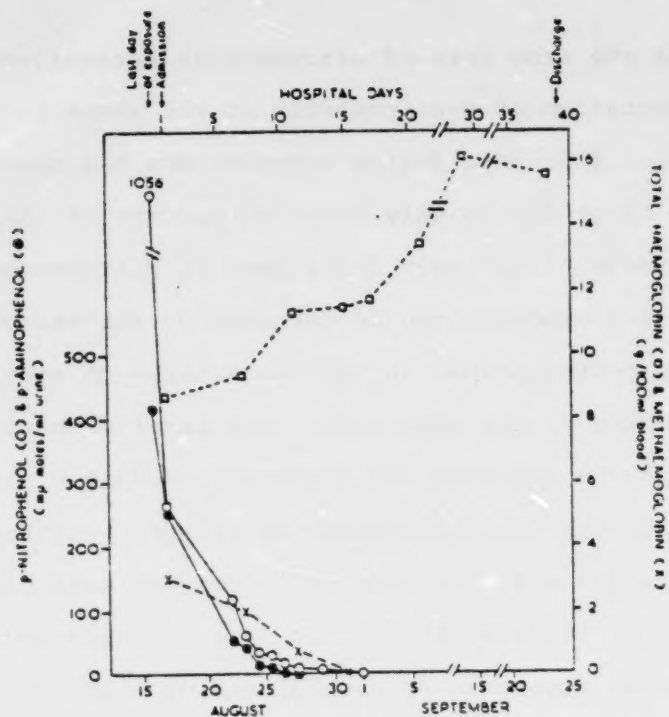


Figure 2. Changes in the levels of total hemoglobin and methaemoglobin in blood and of p-nitrophenol and p-aminophenol in urine. The usual daily volume of urine was about 1 litre.

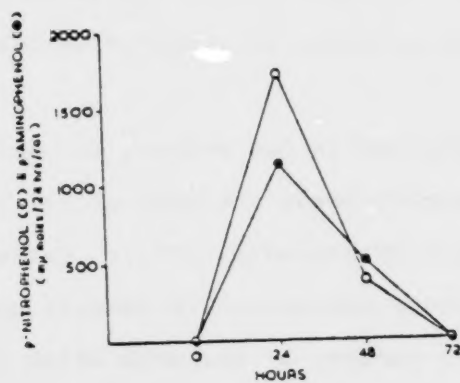


Figure 3. Excretion of p-nitrophenol and p-aminophenol in the urine of rats exposed to nitrobenzene.

Because of the slow rate of nitrobenzene metabolism in man, the concentration of p-nitrophenol in the urine increases for about four days during exposure and the concentration on the first day is only about 40 percent of the peak value. An estimate of the mean daily dose of nitrobenzene in chronic industrial exposure can be obtained by the measurement of urinary p-nitrophenol in specimens taken on each of the last three days of the work week. The level of nitrobenzene exposure can be approximated using the formula $y = 0.18z$, where y is the daily excretion of urinary p-nitrophenol in mg/day and z is the mean daily dose of absorbed nitrobenzene in mg (Piotrowski, 1967). The extended systemic retention and slow excretion of metabolites of nitrobenzene in man is determined by the low rate of metabolic transformation (reduction and hydroxylation) of the nitrobenzene itself. The conjugation and excretion of the metabolites, p-nitrophenol and p-aminophenol, is rapid (Piotrowski, 1977).

The urinary metabolites in man account for only 20 to 30 percent of the nitrobenzene dose; the fate of the rest of the metabolites is not known (Piotrowski, 1977). Parke (1956) studied ^{14}C - nitrobenzene metabolism in rabbits and was able to account for 85 to 90 percent of the dose which was administered by intubation. One percent of the nitrobenzene was exhaled as CO_2 in air, and 0.6 percent was exhaled as unchanged nitrobenzene. Fifty-eight percent of the dose appeared as urinary metabolites, p-aminophenol, nitrophenols, aminophenols, nitrocatechols, and aniline. Thirty percent of

the nitrobenzene was still in the rabbit tissue four to five days after dosing, and nine percent of the nitrobenzene metabolizes was in the feces.

Urinary p-nitrophenol in man is determined after hydrolysis of the conjugated metabolites. Analytical methodology (of which there are several methods) involves removal of interfering color substances, hydrolysis, extraction of p-nitrophenol, re-extraction into an aqueous system, reduction to a p-aminophenol, and reaction to indophenol, which is a blue colored product. The sensitivity is 5 µg per sample (Piotrowski, 1977).

EFFECTS

Acute, Sub-acute, and Chronic Toxicity

Acute exposure to nitrobenzene can occur from accidental or suicidal ingestion of the liquid nitrobenzene or ingestion as false bitter almond oil in food or medicine. Cutaneous absorption causing acute toxic reactions can result from wearing wet, freshly dyed shoes (Levin, 1927), marking ink used on diapers or protective pads (Etteldorf, 1951), use of soap or skin oil containing nitrobenzene (Zeitoun, 1959), or an untreated spill of nitrobenzene on the skin in an industrial plant (Hamilton, 1919). The fatal dose of nitrobenzene in humans varies widely; values from less than 1 ml to over 400 ml have been reported (Wirtschafter and Wolpaw, 1944). Chronic toxic effects in man generally result from industrial exposure to vapors that are absorbed through the lungs or the skin. One case of chronic toxicity was reported in a woman who used nitrobenzene as a cleaning solution for many years (Hamilton, 1919).

Symptoms of chronic occupational nitrobenzene absorption are cyanosis, methemoglobinemia, jaundice, anemia, sulfhemoglobinemia, presence of Heinz bodies in the erythrocytes, dark colored urine, and the presence of nitrobenzene metabolites (e.g. nitrophenol) in the urine (Pacseri and Magos, 1958; Hamilton, 1919; Wuertz, et al. 1964; Browning, 1950; Malden, 1907; Piotrowski, 1967).

The symptoms of dinitrobenzene poisoning include those found in nitrobenzene toxicity as well as abdominal pain, weakness, enlarged liver, and basophilic granulations of red corpuscles (Beritic, 1956; Malden, 1907). Dinitrobenzene poisoning also causes unequal responses in different exposed workers.

The outstanding symptom of acute nitrobenzene poisoning is cyanosis as a result of methemoglobin formation (up to 80 percent) (Piotrowski, 1967). If the cyanosis is severe or prolonged the patient will go into coma and may die. Often anemia is seen a week or two after acute poisoning as a result of the hemolytic effect of nitrobenzene (Stevenson and Forbes, 1942). Suicidal ingestion of nitrobenzene has been reported (Nabarro, 1948; Leinoff, 1936; Myslak, et al. 1971), and it has also been used unsuccessfully to induce abortion (Nabarro, 1948; Dorigan and Hushon, 1976). Harrison (1977) reported a case of poisoning from an aniline-nitrobenzene mixture which was accidentally ingested from a pipette by a chemistry student. The mortality due to ingested nitrobenzene in the above cases was variable, depending on the health

of the patients and the treatments they received. Common treatments include gavage, transfusions, oxygen therapy, methylene blue, ascorbic acid, and toluidine blue. Treatment is usually directed to reduce the methemoglobinemia which is the immediate effect, and often the cause of death in nitrobenzene poisoning. Death has resulted from intake of less than 1 ml of nitrobenzene (Wirtschafter and Wolpaw, 1944).

Some of the reported toxicity values are summarized in Table 4 (Fairchild, 1977). The term LDLo designates the lowest reported lethal dose and TDLo is the lowest published toxic dose.

TABLE 4

Acute Toxicity Values (Fairchild, 1977)

<u>Animal</u>	<u>Route</u>	<u>Toxic Dose</u>
woman	oral	TDLo: 200 mg/kg
human	oral	LDLo: 5 mg/kg
rat	oral	LD50: 640 mg/kg
rat	skin	LD50: 2100 mg/kg
rat	intraper.	LD50: 640 mg/kg
rat	subcutan.	LDLo: 800 mg/kg
mouse	subcutan.	LDLo: 286 mg/kg
dog	oral	LDLo: 750 mg/kg
dog	intraven.	LDLo: 150 mg/kg
cat	oral	LDLo: 2000 mg/kg
cat	skin	LDLo: 25 mg/kg
rabbit	oral	LDLo: 700 mg/kg
rabbit	skin	LDLo: 600 mg/kg
guinea pig	intraper.	LDLo: 500 mg/kg

Aquatic toxicity at 96 hours - 10-100 mg/l (ppm).

Levin (1927) demonstrated in vivo production of methemoglobin by nitrobenzene in dogs, cats, and rats, but not in guinea pigs or rabbits. Dresbach and Chandler (1918) found that nitrobenzene fumes caused cerebellar disturbances in dogs and birds, while blood changes were the principal toxic effects in other mammals they studied. Reddy, et al. (1976) reported a delay in methemoglobin formation in germ free rats by nitrobenzene and postulated that the gut flora of rats was responsible for the reduction (in vivo) and methemoglobin forming capacity of nitrobenzene. Shimkin (1939) measured the toxicity of nitrobenzene in mice when absorbed through the skin. He found the minimum lethal dose to be 0.0004 ml/gm body weight by a subcutaneous route of administration. The nitrobenzene caused respiratory failure, reduction of the white blood cell count, and liver pathology in the mice.

Yamada (1958) did a chronic toxicity study in rabbits that received a subcutaneous dose of 840 mg/kg body weight per day for three months. He found a decrease in erythrocyte number and hemoglobin content early in the exposure. These values increased during the three months but did not return to normal levels. Urinary excretion of detoxification products was variable in the early stages of the exposure, but then all the detoxification reactions (reduction, hydroxylation, and acetylation) were depressed. As a result of these observations, Yamada divided this response in the rabbit into three stages: initial response, resistance, and exhaustion.

The effects of subacute nitrobenzene exposure in rats were studied by Kulinskaya (1974). Vasilenko and Zvezdai (1972) measured blood changes and found sulfhemoglobin formation to be the most regular and persistent change noted. Increased methemoglobin levels with Heinz body formation and anemia were also seen.

The cytotoxicity of nitrobenzene to cultured Erlich-Landschutz diploid (ELD) cells was measured by Holmberg and Malmfors (1974). They found no significant increase in cell injury after five hours incubation with nitrobenzene. However, a 3M nitrobenzene solution reduced cell proliferation by 50 percent in cultured hamster cells (Raleigh, et al. 1973). Nitrobenzene increases oxygen consumption by cultured cells (Biaglow and Jacobson, 1977), and its derivatives are used to sensitize malignant cells in vitro for radiation (Chapman, et al. 1974). They suggest that this effect is due to radical oxidation and increased cellular damage.

Nitrobenzene derivatives have a wide variety of toxic effects. 1-Chloro - 2, 4 dinitrobenzene (DNCB) is a well known skin sensitizer in guinea pigs, mice, and man (Hama-guchi, et al. 1972; Jansen and Bleumink, 1970; Maurer, et al. 1975; Weigand and Gaylor, 1974; Noonan and Halliday, 1978). Cooke, et al. (1968) showed that nitrobenzene derivatives react with cell membranes to alter sodium-potassium conductance, and sometimes affect action potentials of nerve cells.

M-dinitrobenzene is a potent methemoglobin former, and is more toxic than nitrobenzene (Ishihara, et al. 1976; Pankow, et al. 1975). Pentachloronitrobenzene (PCNB) is a common fungicide with varying toxic effects in different mammalian species (Courtney, et al. 1976).

Some of the toxic effects of nitrobenzene are summarized in Table 5 and Apendix A (Dorigan and Hushon, 1976).

TABLE 5

Toxicological Effects of Nitrobenzene

<u>Organism</u>	<u>Route</u>	<u>Exposure</u>	<u>Exposure Time</u>	<u>Response</u>	<u>Source</u>
Human	Inhalation	0.2-0.5 mg/l (40-100 ppm)	ca. 6 hrs.	Slight effects, e.g. headache, fatigue.	von Oettingen, 1941.
	Inhalation	0.129 mg/m ³	-	Threshold level for electroencephalograph disturbance.	Andreeshecheva, 1964.
	Inhalation	6-30 µg/l	6 hrs.	Retained 80% of vapor in lungs. Urinary excretion of p-nitrophenol (max. in 2 hrs. still detected after 100 hrs.).	Salmowa, et al. 1963.
Rabbit	Oral	333 ml	Single	Max. dose with recovery reported (following severe symptoms)	von Oettingen, 1941.
	Oral	0.4 ml	Single	Min. lethal dose reported.	von Oettingen, 1941
	Cutaneous absorption	0.7 gm/kg	Single (?)	After 52 hrs.: lethal	von Oettingen, 1941.
	Oral	700 mg/kg	Single	Lethal dose	Stecher, 1968
	Oral	600 mg	Single	Dizziness, loss of reflexes, methemoglobinemia, congestion of brain tissue-12 hrs.-death.	Chandler, 1919.
	Oral	50 mg/kg	Single	Tissue degeneration, especially heart, liver, kidney.	Papageorgiou & Argoudelis, 1973.
	Oral				

TABLE 5 (Continued)

<u>Organism</u>	<u>Route</u>	<u>Exposure</u>	<u>Exposure Time</u>	<u>Response</u>	<u>Source</u>
Guinea pig	Inhalation	Saturated air (0.04 vol. %)	2-5 hrs.	Death following tremors, paralysis of hind legs.	Chandler, 1919.
	Oral	ca. 3 gm	Single	0.5 hrs.: tremors, faint heartbeats, labored respiration 2 hrs.: death.	Chandler, 1919.
	Oral	ca 1.2 gm	Single	Immediately motionless, then complete recovery.	Chandler, 1919.
Rat	Inhalation	5 mg/m ³	8 hrs.	Metabolites excreted in 3 days.	Ikeda and Kita, 1964
	Inhalation	ca. 0.03mg/m ³	Daily up to 98 days	Increased ability to form sulfhemoglobin in preference to methemoglobin.	Andreeshcheva, 1970.
	Inhalation	0.06-0.1 mg/m ³	70-82 days	Cerebellar disturbances, inflamed internal organs.	Khanin, 1969.
Cat	Inhalation	0.008 mg/m ³	73 days	No effect.	Andreeshcheva, 1970.
	Oral	0.6 gm/kg	Single	LD ₅₀	Smyth, et al. 1969
	Inhalation	Saturated air (0.04 vol. %)	2-5 hrs.	Death following tremors, paralysis of hind legs.	Chandler, 1919.
Dog	Oral	2.4 gm	Single	Death in 12-24 hrs.	von Oettingen, 1941; Chandler, 1919.
	Oral	2.4 gm	Single	1 hr.: vomiting, then sleepy continuing for 6 hrs. 104 hrs.: death.	Chandler, 1919.

Synergism and/or Antagonism

Alcohol has a synergistic effect on nitrobenzene poisoning. Ingestion of an alcoholic beverage has induced immediate acute toxic symptoms, including coma, in a worker recovered from the effects of chronic nitrobenzene exposure. Alcohol ingestion or chronic alcoholism can lower the lethal or toxic dose of nitrobenzene (Dorigan and Hushon, 1976). In subchronic dinitrobenzene poisoning, drinking of one beer or exposure to sun can bring on an acute crisis as late as six weeks after the disappearance of other symptoms (Rejsek, 1947). Therefore, workers exposed to subacute levels of nitrobenzene or dinitrobenzene should be aware of the possible deleterious synergistic effects of alcoholic beverages and sunlight.

Smyth, et al. (1969) studied the synergistic action between nitrobenzene and 27 other industrial chemicals by intubation in rats. Most of the compounds tested did not alter the LD50. In another study, ingestion of 2 to 20 ml of ethanol increased the severity of reaction to a 0.1 ml intravenous dose of nitrobenzene in rabbits. This observation agrees with the clinical data on the synergism of ethanol and nitrobenzene (Dorigan and Hushon, 1976).

Kaplan, et al. (1974) studied the effect of caffeine, an inducer of microsomal enzymes, on methemoglobin formation by nitrobenzene in rats. Methemoglobin was formed and then decreased in induced animals. The increased microsomal enzyme level increased the rate of metabolism and excretion of nitrobenzene and thus caused a rapid decline of methemoglobin levels.

Teratogenecity

There is a paucity of information on the teratogenic effects of nitrobenzene. In one study (Kazanina, 1968b), 125 mg/kg was administered subcutaneously to pregnant rats during preimplantation and placentation periods. Delay of embryogenesis, alteration of normal placentation, and abnormalities in the fetuses were observed. Gross morphogenic defects were seen in four of 30 fetuses examined.

Changes in the tissues of the chorion and placenta of pregnant women who worked in the production of a rubber catalyst that used nitrobenzene were observed. No mention was made of the effects on fetal development or viability (Dorigan and Hushon, 1976). Menstrual disturbances after chronic nitrobenzene exposure have been reported.

Garg, et al. (1976) tested substituted nitrobenzene derivatives for their ability to inhibit pregnancy in albino rats. Two of the compounds tested (p-methoxy and p-ethoxy derivatives) inhibited implantation and pregnancy 100 percent when administered on days one through seven after impregnation.

The available data, although sketchy, indicate that women who are or wish to become pregnant should avoid exposure to nitrobenzene. Further studies of nitrobenzene teratogenicity in mammals are needed.

Mutagenicity

Chiu, et al. (1978) tested nitrobenzene and 53 commercially available ion, heterocyclic + aliphatic nitro

compounds for mutagenicity using the Ames Salmonella typhimurium TA 98 and TA 100. They reported that 34 of the 53 compounds tested were mutagenic. Nitrobenzene was not found to be mutagenic.

Trinitrobenzene was mutagenic in two in vitro assays, the Ames Salmonella microsome assay, and the mitotic recombination assay in yeast (Simmon, 1977). Other nitrobenzene derivatives have demonstrated mutagenicity in in vitro assays, so that the mutagenicity of nitrobenzene is still in question and additional work is needed in this area.

Carcinogenicity

The available literature does not demonstrate the carcinogenicity of nitrobenzene, although it is suspect (Dorigan and Hushon, 1976). This is another aspect of nitrobenzene effects that needs more research.

Some nitrobenzene derivatives have demonstrated carcinogenic capacities. Pentachloronitrobenzene (PCNB) induced hepatomas and papillomas in mice (Courtney, et al. 1976).

1 - Fluoro - 2, 4 - dinitrobenzene (DNFB) was demonstrated by Bock, et al. (1969) to be a promoter of skin tumors in mice, although it does not induce them when administered alone.

CRITERION FORMULATION

Existing Guidelines and Standards

The maximum allowable concentration of nitrobenzene in air in industrial plants is 5 mg/m^3 . This value was set by the joint ILO/WHO Committee on Occupational Health in 1975 (Goldstein, 1975). The OSHA (Occupational Safety and Health Administration) standard for nitrobenzene in air is 5 mg/m^3 (1 ppm) set in 1977 (Am. Conf. Gov. Ind. Hyg., 1977). This is also the threshold limit value (TLV) in Germany and Sweden while the TLV in the USSR is 3 mg/m^3 (Dorigan and Hushon, 1976).

There are no standards for nitrobenzene levels in water. Nitrobenzene was not listed among the substances for which a maximum concentration has been set.

Current Levels of Exposure

A worker exposed to the current occupational standard of 5 mg/m^3 (1 ppm) nitrobenzene for an eight-hour work day would absorb approximately 24 mg by inhalation and 9 mg cutaneously. The maximum eight-hour uptake would be 33 mg, which is less than the "reasonable safe" level of 35 mg/day (Dorigan and Hushon, 1976). Doses of up to 70 mg/day have been reported for factory workers and up to 80 mg/day have been reported in a dye stuff factory in England (Dorigan and Hushon, 1976, citing Piotrowski, 1967).

Nitrobenzene can be a contaminant in industrial waste water, and companies utilizing or making nitrobenzene are required to monitor its level in their effluent waste (Pierce, 1979). The minimum detectable level of nitrobenzene in

drinking water by gas chromatography is 0.7 ng (Austern, et al. 1975).

Nitrobenzene may be vented to the atmosphere. The vents are usually equipped with absorbers or scrubbers, but some nitrobenzene vapors can escape. Atmospheric nitrobenzene levels outside a plant are not monitored by industry. Since inner plant levels are below the TLV of 5 mg/m^3 (1 ppm) and nitrobenzene vapors accumulate at the floor level due to their high density, the external air nitrobenzene concentrations are expected to be very low (Dorigan and Hushon, 1976).

Special Group at Risk

Workers in plants producing or using nitrobenzene have the greatest risk of toxic exposure. At the current TLV level of 5 mg/m^3 (1 ppm) a worker could absorb as much as 33 mg/day. This is enough to produce symptoms of chronic toxicity in some susceptible individuals (Dorigan and Hushon, 1976). The amount of nitrobenzene absorbed by a worker via inhalation and cutaneous absorption can be estimated from the level of total (free and conjugated) p-nitrophenol in urine as described by Piotrowski (1977).

Due to the current widespread use of disposable diapers and underpads in hospitals, nitrobenzene poisoning in infants from laundry marking dyes is no longer a problem.

Pregnant women may be especially at risk with respect to nitrobenzene as with many other chemical compounds, due to transplacental passage of the agent. Individuals with glucose-6-phosphate dehydrogenase deficiency may also be

special risk groups (Calabrese, et al. 1977; Djerassi, et al. 1975). Additionally, because alcohol ingestion or chronic alcoholism can lower the lethal or toxic dose of nitrobenzene (Rejsek, 1947; von Oettingen, 1941), individuals consuming alcoholic beverages may be at risk.

Basis and Derivation of Criterion

Because there are little or no data available on the toxicity of nitrobenzene ingested in drinking water, or on the teratogenic, mutagenic, or carcinogenic effects of nitrobenzene in general, experimental testing is necessary before an oral ingestion based criterion can be derived. It is recommended that testing in these areas of toxicity be implemented so that the effects of nitrobenzene on mammals may be better understood.

Using the methodology of Stokinger and Woodward (1958), a water quality criteria (WQC) is derived using the organoleptic level and the TLV.

Organoleptic Level: minimum detectable odor level in water is $0.03 \text{ mg/l} = 30 \text{ } \mu\text{g/l}$.

Assuming a daily intake of 2 liters of water, the total intake of nitrobenzene based on this criteria would be 60 micrograms/day. Recommended WQC = $30 \text{ } \mu\text{g/l}$.

A calculation of the percentage of exposure attributable to fish and shellfish products is not applicable to a criterion based upon organoleptic effects. Since an organoleptic effect is not based on a toxicological assessment, it

would be inappropriate to apportion a percentage of exposure to the consumption of toxicologically contaminated fish.

TLV: TLV = 5 mg/m³; air intake = 10 m³/day; assume 80 percent absorption:

$$(5 \text{ mg/m}^3) \times (10 \text{ m}^3/\text{day}) \times (0.8) = 40 \text{ mg/day}$$

average over seven days:

$$40 \text{ mg/day} \times 5/7 = 29 \text{ mg/day}$$

Assuming 100 percent gastrointestinal absorption of nitrobenzene and consuming 2 liters of water daily and 18.7 grams of contaminated fish having a bioconcentration factor of 4.3, would result in a maximum permissible concentration of 13.9 mg/l for the ingested water:

$$\frac{29 \text{ mg/day}}{2 \text{ liters} + (4.3 \times 0.0187) \times 1.0} = 13.9 \text{ mg/l}$$

$$\text{WQC using TLV} = 13.9 \text{ mg/l}$$

Since the WQC using TLV is well above the detectable odor level of nitrobenzene, water containing this concentration of nitrobenzene would not be esthetically acceptable for drinking. Even though the limitations of using organoleptic data as a basis for establishing a WQC are recognized, it is recommended that a WQC of 30 µg/l be established at the present time. This level may be altered as more data are developed upon which to calculate a WQC.

The analysis and recommendations generated in this document are based on the literature available to date. If future reports indicate that nitrobenzene may be carcinogenic, mutagenic or teratogenic, a reassessment of the WQC will be necessary.

APPENDIX A

Toxiological Effects of Nitrobenzene

<u>Organism</u>	<u>Route</u>	<u>Exposure</u>	<u>Exposure Time</u>	<u>Response</u>
Human	Inhalation	-	8 hrs./day for 17 mos. factory worker	Cyanosis, headache, fatigue methemoglobinemia (Ikeda and Kita, 1964).
	Inhalation	Poor ventilation	8 hrs./day for 1.5 mos. factory worker paint firm	Cyanosis, headache, fatigue, methemoglobinemia, liver damage, hypotension (Ikeda and Kita, 1964).
			8 hrs./day for 4.5 mos.	Above plus: liver and spleen enlarged and tender, hyperalgesia in extremities (Ikeda and Kita, 1964).
	Inhalation	0.2-0.5 mg/l (40-100 ppm)	ca. 6 hrs.	Slight effects, e.g. headache, fatigue (von Oettingen, 1941).
	Inhalation	0.129 mg/m ³	-	Threshold level for electroencephalograph disturbance (Andreeshcheva, 1964).
	Inhalation	"Large" amounts poor ventilation		Hospitalized: 2 - vertigo, coma, cyanosis 3 - labored breathing, urine with 7 - almond odor, methemoglobinemia recovery after 1 mo. (Ravault, et al. 1946).
	Inhalation	Acute	-	Burning throat, nausea, vomiting, gastrointestinal disturbances, cold skin, livid face, cyanosis (von Oettingen, 1941).

APPENDIX A (Continued)

<u>Organism</u>	<u>Route</u>	<u>Exposure</u>	<u>Exposure Time</u>	<u>Response</u>
Human	Inhalation	-	Nitrobenzene factory worker	Intermittent symptoms: cyanosis, pallor and jaundice, pharyngeal congestion, headache, changes in blood cell composition (increased polynuclears and eosinophils (von Oettingen, 1941).
	Inhalation	6-30 µg/l	6 hrs.	Retained 80% of vapor in lungs, urinary excretion of p-nitrophenol (maximum in 2 hrs., still detected after 100 hrs.) (Salmowa, et al. 1963).
	Inhalation	-	Factory worker (rubber accelerator)	Pregnant women: thickening of tissue in blood vessels, decreased placental absorption, necrosis in placental tissue (Ferster, 1970).
	Inhalation	-	Factory worker (glass, porcelain)	Changes in bone marrow, increased lymphoid cell production, impairment of copper metabolism and certain iron-containing enzymes (Yordanova, et al. 1971).
	Inhalation	-	Industrial exposure	Disturbance of motor impulses (Zenk, 1970).
	Inhalation	Acute	Factory worker (filled containers with nitrobenzene)	14 days: cyanosis, headache, backache, stomach ache, vomiting ca. 21 days: drank beer and fell unconscious, cyanosis, dilated pupils, retarded respiration, weak pulse 1 yr.: intelligence dimmed 2 yrs.: emaciated, atrophied muscles 3 yrs.: memory failed 6 yrs.: loss of perception of time and space (Korsakoff's syndrome) (Chandler, 1919).

APPENDIX A (Continued)

<u>Organism</u>	<u>Route</u>	<u>Exposure</u>	<u>Exposure Time</u>	<u>Response</u>
Human	Cutaneous absorption	Dye used in diaper stamps	-	Babies: cyanosis, rapid pulse, shallow respiration, vomiting, convulsions, recovery in 24 hrs. (von Oettingen, 1941).
	Cutaneous absorption	Shoe dye	ca. 7 hrs.	Unconsciousness after consumption of alcohol beverages, death (Chandler, 1919).
	Cutaneous absorption	0.5% by weight in paper	- (Handled carbon paper)	Dermatitis (Calan and Connor, 1972).
	Oral	-	From human milk	Nurselings became cyanotic, recovery in 24 hrs. (mothers ate almond cake artificially flavored with nitrobenzene) (Dollinger, 1949).
Rabbit	Oral	333 ml	Single	Maximum dose with recovery reported following severe symptoms (von Oettingen, 1941).
	Oral	0.4 ml	Single	Minimum lethal dose reported (von Oettingen, 1941).
	Subcutaneous injection	0.8 mg/kg	Daily	Maximum dose not causing death (Yamada, 1958).
	Subcutaneous injection	10-14 mg/kg	Single	Minimum dose producing observable effects; slow and lasting methemoglobinemia (von Oettingen, 1941).
	Cutaneous absorption	0.7 gm/kg	Single	After 52 hrs.: lethal (von Oettingen, 1941).
	Intraperitoneal injection	0.5gm/kg	Single	Reduced blood pressure and myocardial glycogen level (Labunski, 1972).

APPENDIX A (Continued)

<u>Organism</u>	<u>Route</u>	<u>Exposure</u>	<u>Exposure Time</u>	<u>Response</u>
Rabbit	Intravenous	0.1 gm	Daily or every 5 days	Simultaneous doses of 2-20 ml ethanol increased severity of poisoning (Matsumara and Yoshida, 1959).
	Oral	9 gm	4 doses, one every 15 minutes	Convulsions, death (von Oettingen, 1941; Chandler, 1919).
	Oral	4.8 gm	Single	Lethal instantly (von Oettingen, 1941; Chandler, 1919).
	Oral	700 mg/kg	Single	Lethal dose (Stecher, 1968).
	Oral	600 mg	Single	Dizziness, loss of reflexes, methemoglobinemia, congestion of brain tissue - 12 hrs. - death (Chandler, 1919).
	Oral	300 mg	Single	Fatigue for 1 week (Parke, 1956).
	Oral	50 mg/kg	Single	Tissue degeneration, especially heart, liver, kidney (Papageorgiou and Argoudelis, 1973).
	Oral	1 mg/kg	Single	Lowered hemoglobin, erythrocytes and lymphocytes; increased leucocytes (Kazakova, 1956).
	Oral	0.1 mg/kg	Single	Threshold toxic dose (Kazakova, 1956).
	Inhalation	Saturated air (0.04 vol. %)	2-5 hrs.	Death following tremors, paralysis of hind legs (Chandler, 1919).
Guinea pig	Inhalation		2-3 hrs.	Death (Chandler, 1919).
	Subcutaneous	0.2 gm/kg	Every other day for 6 mos.	Hemolytic anemia, loss of weight, decreased motor activity, fluxes in urinary excretion of 17-hydroxy-corticosteroids (Porter-Silber chromogens) (Makotchenko and Akhmetov, 1972).

APPENDIX A (Continued)

<u>Organism</u>	<u>Route</u>	<u>Exposure</u>	<u>Exposure Time</u>	<u>Response</u>
Guinea pig	Oral	ca. 3 gm	Single	0.5 hrs.: tremors, faint heartbeats, labored respiration 2 hrs.: death (Chandler, 1919).
	Oral	ca. 1.2 gm	Single	Immediately motionless, then complete recovery (Chandler, 1919).
	Oral	50 mg/kg	1 year	Tissue degeneration, especially heart, liver, kidney (Kazakova, 1956).
	Oral	1 mg/kg	Single	Lowered hemoglobin, erythrocytes, lymphocytes; increased leucocytes (Kazakova, 1956).
Rat	Oral	0.1 mg/kg	Single	Threshold toxic dose (Kazakova, 1956).
	Inhalation	5 mg/m ³	8 hrs.	Metabolites excreted in 3 days (Ikeda and Kita, 1964).
	Inhalation	ca. 0.03 mg/m ³	Daily, up to 98 days	Increased ability to form sulfhemoglobin in preference to methemoglobin (Andreeshcheva, 1970).
	Inhalation	0.006-0.1 mg/m ³	70-82 days	Cerebellar disturbances, inflamed internal organs (Khanin, 1969).
	Inhalation	0.008 mg/m ³	73 days	No effect (Andreeshcheva, 1964).
	Oral	0.6 gm/kg	Single	LD ₅₀ (Smyth, et al. (1969).
	Intraperitoneal injection	0.8gm/kg	Single	Lethal (Magos and Sziza, 1958).

APPENDIX A (Continued)

<u>Organism</u>	<u>Route</u>	<u>Exposure</u>	<u>Exposure Time</u>	<u>Response</u>
Rat	Subcutaneous injection	640 mg/kg	Single	Blood catalase activity decreased continuously over 96 hrs. (Goldstein and Popovici, 1959).
	Subcutaneous injection	300 mg/kg	Single	LD (14 days) - methemoglobinemia, anemia, sulfhemoglobinemia (Brown, et al. 1975).
	Subcutaneous injection	200 mg/kg or 100 mg/kg	Single	Methemoglobinemia, sulfhemoglobinemia, anemia (Zvezdai, 1972).
	Subcutaneous injection	125 mg/kg	Daily for 10 days	Delayed embryogenesis, abnormal fetal development and embryo death, changes in polysaccharide composition of placenta (Kazanina, 1967, 1968a,c).
	Subcutaneous injection	100-200 mg/kg	Single	Sulfhemoglobin (most regular and persistent form of hemoglobin) nitroxyhemoglobin, increased methemoglobin (Vasilenko and Zvezdai, 1972).
Mouse	Cutaneous absorption	480 mg/kg	-	30 min.: prostrate, motionless 24 hrs.: death (von Oettingen, 1941).
	Intraperitoneal injection	1.23 gm/kg	Single	40 min.: 67% dead (Smith, et al. 1967).
	Intraperitoneal injection	1 gm/kg	Single	10-15 min.: incoordination, comatose, shallow respiration Several hrs.: regained coordination Immediately before death: lost coordination again, respiratory arrest 48 hrs.: death (Smith, et al. 1967).

APPENDIX A (Continued)

<u>Organism</u>	<u>Route</u>	<u>Exposure</u>	<u>Exposure Time</u>	<u>Response</u>
Mouse	Intraperitoneal injection	20 mg/kg	Single	Lethal dose (Brown, et al. 1975).
	Intraperitoneal injection	12.3 mg/kg	Single	10 min.: 4.2% methemoglobin formed (Smith, et al. 1967).
Cat	Inhalation	Saturated air (0.04 vol. %)	2-5 hrs.	Death following tremors, paralysis of hind legs (Chandler, 1919).
	Inhalation	-	2-3 hrs.	Death
	Oral	2.4 gm	Single	Death in 12-24 hrs. (von Oettingen, 1941; Chandler, 1919).
Dog	Inhalation	"Thick vapor"	1.5 hrs.	Complete anesthesia and sleep (Chandler, 1919).
	Intravenous injection	0.15-0.25 gm/kg	Single	Minimum lethal dose - lowered blood pressure, pulse rate increased then decreased respiration stimulated until paralyzed (von Oettingen, 1941).
	Oral	28.8 gm plus 6 gm	2 doses, 0.5 hrs. apart	Immediate: agitation, then motionless 1 hr.: convulsions, then motionless 4.5 hrs.: tremors, hind legs paralyzed 18 hrs.: death (Chandler, 1919).
	Oral	24 gm	Single	Few hrs.: "stupid" 12 hrs.: deep coma, slow respiration, lowered skin temperature, stomach strongly alkaline (Chandler, 1919).

APPENDIX A (Continued)

<u>Organism</u>	<u>Route</u>	<u>Exposure</u>	<u>Exposure Time</u>	<u>Response</u>
Dog	Oral	2.4 gm	Single	1 hr.: vomiting, then sleep continuing for 6 hrs. 6 hrs.: appeared normal 15-68 hrs.: rigid muscles 104 hrs.: death (Chandler, 1919). Minimum lethal dose (von Oettingen, 1941). Salivation, unrest, dizziness, tremors, increased pulse rate, sometimes convulsions (Chandler, 1919). Formed methemoglobin continuously at "certain" concentration (Hashimoto, 1958). Unsteady gait, recovery (Chandler, 1919). Immediately unconscious 12 hrs.: death (Chandler, 1919). No effects Death (Chandler, 1919).
C 1 4 6	Oral	0.75-1.0 gm/kg	Single	
	Oral	0.5-0.7 gm/kg	Single	
	Oral	-	Daily	
Chicken	Oral	1.2 gm	Single	
	Oral	2.4 gm	Single	
Pigeon	Inhalation	-	1 hr. 2-3 hrs.	

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